SpectraMax® Paradigm®
Fluorescence Polarization (FP) Detection Cartridge

User Guide

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Safety Information

WARNING! If the equipment is used in a manner not specified by Molecular Devices, the protection provided by the equipment may be impaired.

Warning and Caution Definitions

All Warnings and Cautions in this document include an exclamation point, a lightning bolt, or a light burst symbol framed within a triangle.

The exclamation point symbol is an international symbol which serves as a reminder that all safety instructions should be read and understood before installation, use, maintenance, and servicing is attempted.

WARNING! A WARNING calls attention to a condition or possible situation that could cause injury to the operator.

CAUTION! A CAUTION calls attention to a condition or possible situation that could damage or destroy the product or the operator’s work.

When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies.
Contact a service engineer for any servicing of equipment requiring the removal of covers or panels.

**High Voltage**

**WARNING! HIGH VOLTAGE!** This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) wall outlets. Use only multiplug power strips provided by the manufacturer.

**Disposal of Electronic Equipment**

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- the device was put on the European Market after August 13, 2005.
- the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Molecular Devices office for the proper decontamination information and take back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.
Laser Light

**WARNING!** This symbol indicates that a potential hazard to personal safety exists from a laser source. When this symbol is displayed in this manual, pay special attention to the specific safety information associated with the symbol.

Laser Specifications

The SpectraMax® Paradigm® Multi-Mode Detection Platform is rated a Class 1 Laser Product because it houses one or more Laser Modules whereas the laser light is not accessible.

![Class 1 Laser Product Label](image)

*Figure 2-1* Label on Rear of Instrument
The embedded Laser Module inside the SpectraMax Paradigm Multi-Mode Detection Platform base instrument is used for the plate height detection and has the following specifications:

- Laser type: diode laser
- Wavelength: 650 nm
- Max. output power: 2 mW, cw
- Laser Class: Class 2M (IEC60825-1, ed. 1.2: 2001)
- Fan angle: 55° ±5°

The embedded laser module is ON, only if the microplate chamber flap is closed (hardware interlock). The user or the service engineer is not exposed to any radiation from the embedded laser module during operation, maintenance, or service. The closed microplate chamber acts as the protective housing.

**Laser or Laser Diodes Inside the Cartridge Modules**

Various cartridge modules can have a Laser or Laser Diode (up to Laser Class IV) inside the cartridge modules.

The SpectraMax Paradigm Multi-Mode Detection Platform is equipped with a redundant laser safety system. Therefore, the user or the service engineer is not exposed to any radiation of the embedded laser during operation, maintenance, or service.
Chemical and Biological Safety

Normal operation of the SpectraMax Paradigm Multi-Mode Detection Platform may involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility’s waste disposal procedures.
- Operate the SpectraMax Paradigm Multi-Mode Detection Platform in accordance with the instructions outlined in this manual, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

**Note:** Observe all warnings and cautions listed for any external devices attached or used during operation of the Instrument Name. Refer to applicable external device user guides for operating procedures of that device.
Moving Parts

To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the SpectraMax Paradigm Multi-Mode Detection Platform.
- Keep the SpectraMax Paradigm Multi-Mode Detection Platform work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user guide for the SpectraMax Paradigm Multi-Mode Detection Platform. Prior to cleaning equipment that has been exposed to hazardous material:

- Appropriate Chemical and Biological Safety personnel should be contacted.
- The Chemical and Biological Safety information contained in this user guide should be reviewed.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

Note: It is your responsibility to decontaminate components of the Instrument Name before requesting service by a service engineer or returning parts to Molecular Devices for repair. Molecular Devices will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.
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Overview

The SpectraMax® Paradigm® Fluorescence Polarization (FP) Detection Cartridge is a detection cartridge for use with the SpectraMax Paradigm Multi-Mode Detection Platform. Installing the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge enables fluorescence polarization detection methods for specific labels, depending on the cartridge. SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridges are available for the following labels:

- Fluorescein (PN 0200-7009)
- Rhodamine (PN 0200-7010)

A detection cartridge contains the light source, optics, and electrical components needed to perform specific measurement modes or for specific applications. The SpectraMax Paradigm Multi-Mode Detection Platform requires these detection cartridges to enable different measurement modes.

The SpectraMax Paradigm Multi-Mode Detection Platform is a modular multi-mode microplate detector. User-installable and removeable detection cartridges allow the detector to be configured for specific applications and easily expand the capabilities of the detector at any time.

The SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge is a single slot detection cartridge and occupies one (1) slot. It may be installed in the top or bottom read detection cartridge transport. Refer to “Installing Detection Cartridges” in the SpectraMax Paradigm Multi-Mode Detection Platform User Guide, for installation instructions.

Note: It is recommended to install the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge in the top read detection cartridge transport.
About Fluorescence Polarization

Fluorescence polarization (FP) is a measurement technique that uses linearly polarized excitation light. The fluorescence emission is measured behind polarizing optics that filter emission light parallel and perpendicular to the polarization plane of the excitation light.

The SpectraMax Paradigm Multi-Mode Detection Platform incorporates a dual emission design to measure both emission channels (parallel and perpendicular) simultaneously, improving precision and reducing read times when measuring two different emission wavelengths.

Figure 1-1 on page 13 depicts an overview of FP. The process can be described as follows:

1. Excitation light is linearly polarized (EX polar) and reflected into the sample by a beamsplitter (B). Labeled ligands oriented as shown by the dipole (represented in the figure as an “I”) in sample para are most easily excited.

2. Nanoseconds after excitation, the fluorophores emit energy with a wavelength greater than the excitation wavelength which is measured as emission. The emission may be in any direction, but can be summarized by three extreme cases as shown in Figure 1-1 on page 13:
   - sample para displays the case in which the labeled ligand does not move at all. In this case, the emission is in the same direction as, or parallel to, the excitation. Most likely indicates that the ligand is bound to a larger, less mobile molecule. This leads to high polarization data.
   - sample perp displays the case in which the labeled ligand is highly mobile. In the short time between excitation and emission, the fluorophore dipole has rotated in the horizontal plane by 90 degrees. As a result, the emission is perpendicular to the excitation, and is most likely caused by unbound ligand. This leads to low polarization data.
• **sample undetected** displays the case in which the labeled ligand is highly mobile, but rotates 90 degrees out of the measurement plane. The fluorescence emission in this case is undetected. The probability of occurrence is the same as for **sample perp.**

**Figure 1-1** Simplified diagram of fluorescence polarization measurement
The cases shown in Figure 1-1 on page 13 are for illustrative purposes. In an FP assay, having signal in only one channel (parallel or perpendicular) does not occur for the following reasons:

- The decay of a fluorophor label follows exponential decay, but the emission for some individual fluorophores may occur earlier or later than the fluorescence lifetime, which is a statistical average.
- The fluorophores are randomly oriented in solution. Thus, any dipole orientation is possible. The fluorophore in these cases contributes to both the parallel and perpendicular emissions proportionally based on its orientation.

**Applications of Fluorescence Polarization**

Fluorescence polarization (FP) measurements provide information on molecular orientation and mobility, and is typically used to quantify the success of a binding reaction between a smaller labeled ligand and a binding site at a much larger or immobilized molecule. FP can also be used to quantify the dissociation or cleavage of the labeled ligand from a binding site.

FP is a homogeneous microplate assay technique and requires only mixing and measuring—no wash steps are required as in an ELISA. It can also be miniaturized, which makes it useful for high-throughput screening applications.
Analyzing Fluorescence Polarization Data

Fluorescence polarization (FP) results in two sets of fluorescence raw data which are reduced to a single set of FP values.

FP assays in microplates are typically designed with two control samples:

- LOW control sample—minimal polarization value resulting from unbound labeled ligand only
- HIGH control sample—maximum polarization value resulting from bound labeled ligand only

The FP data for a sample is evaluated based on its relative position between the low and high control values. Total intensity may also be determined from the raw data and is proportional to the amount of label in a sample.

Analyzing and interpreting FP data typically consists of the following:

- Blank Correction on page 15
- Data Reduction on page 16
- Data Qualification and Validation on page 18

Blank Correction

Many FP assays use small fluorescent label concentrations in the lower nM range. In this range, blank controls become significant when compared to samples. In order to make FP values independent from label concentration and instrument variations, raw data for parallel and perpendicular values are corrected for blanks prior to data reduction:

- \( I_{\text{para}} = \text{raw}_{\text{para}} - \text{blank}_{\text{para}} \)
- \( I_{\text{perp}} = \text{raw}_{\text{perp}} - \text{blank}_{\text{perp}} \)

For improved results, it is recommended to run replicates for all blanks, controls, and samples. In this case, the blank value subtracted is the average value of all blanks. Multi-Mode Analysis Software performs automatic blank averaging and reduction if blanks are defined on the plate layout in the protocol. Refer to “Creating Protocols” in the Multi-Mode Analysis Software User Guide.
Data Reduction

The FP data reduction receives two sets of fluorescence raw data as an input and generates a single set of FP data output. There are two alternative scales commonly used to measure FP. Both scales can be uniquely transformed into each other:

- Milli Polarization units (typically ranging from 0 to 500mP)
- Anisotropy units (ranging from 0 to 0.400, accordingly).

The formula for converting raw data to polarization data (in mP units) is as follows:

\[
P[mP] = \frac{I[\text{para}] - GI[\text{perp}]}{I[\text{para}] + GI[\text{perp}]} \times 1000 \tag{equation 1}
\]

Anisotropy units may also be determined using the relationship between mP and anisotropy:

\[
Anisotropy = \frac{2P[mP]}{1000} = \frac{3 - P[mP]}{1000} \tag{equation 2}
\]

The G-Factor in (equation 1) corrects blanked raw data for differences in the detection efficiencies for parallel and perpendicular emission from the sample. Main contributions to the G-factor are optical specifications of beamsplitters and mirrors between the sample and the PMTs. The G-factor may also be affected by the emission spectrums of the fluorophores and the emission filters used. For example, using fluorescein or the Pan Vera Green low polarization standard (Invitrogen P-3088) results in different G-Factors. The G-Factor is on the order of one. Deviations from unity (G = 1) do not affect the quality of the resulting FP data.
The value of the G-factor is determined by solving (equation 1) for G and applying the formula to the low control. The G-factor can then be described by the equation below:

\[
G = \frac{1 - \frac{P[\text{low}]}{1000}}{1 + \frac{P[\text{low}]}{1000}} \times \frac{I[\text{para}[\text{low}]]}{I[\text{perp}[\text{low}]]}
\]  

(equation 3)

**Note:** The G-Factor is automatically calculated in the G-factor_FP Fluorescein and G-factor_FP Rhodamine template protocols enabled by the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge.

The low polarization control may be addressed with a value determined on a calibrated cuvette-based fluorometer. The value may be also found in the literature, or provided by an assay manufacturer. In principle, an arbitrary value could be defined. Using the above equation (equation 3) for the G-factor is equivalent to fitting the G-factor value such that the low polarization control attains the desired value. When ignoring the actual G-factor (assuming G=1), the polarization values are shifted (values may be less than zero, or much greater than expected). Normally the low polarization control is used for this calibration step, because its polarization value is often close to zero FP units, while the assay window (difference from the low to the high polarization value) depends on the assay (mainly the relative size of the bound complex to the unbound ligand).

For monitoring the assay preparation, the total intensity can be retrieved from the raw data. Total intensity is proportional to the amount of label in a sample. Total intensity does not give information on binding success.
The total intensity of the samples can be calculated using the following formula:

\[ \text{TotalIntensity} = I_{\text{para}} + 2GI_{\text{perp}} \]  

(equation 4)

The factor of two compensates for the undetected sample which is not measured by either the parallel or perpendicular emission channels (see Figure 1-1 on page 13).

**Data Qualification and Validation**

When validating the data of an FP measurement and the assay, there are two factors to look at: the precision value, and the Z’ parameter.

The FP precision value is a measure of replicate uniformity determined by the standard deviation of replicates at a label concentration of 1 nM. Since the precision of a measured signal also depends on the read time, the read time must also be specified. A longer read time leads to a lower (better) precision value.

Z’ is a qualification parameter that can be used to validate the results of a measurement. It is a function of assay resolution, which is a ratio of the average standard deviation of high and low control replicates (S) to the assay window (W)—the difference between the average value of the high controls and the average value of the low controls.

\[ Z' = 1 - 6\frac{S}{W} \]  

(equation 5)

The assay window is dependent on the fluorophore lifetime and relative size of the receptor to the ligand. Precision values are better (lower) at higher signals, which normally come from higher label concentrations.

For a given assay window W, Z’ is a downward sloping linear function. That is, as precision values get higher (worse), the Z’ value gets lower (worse). Thus, to get a better Z’ value, the precision must be improved.
As was discussed earlier, precision is dependent upon assay characteristics (sample volume, label concentration) and read time. In many cases, the assay characteristics are defined and cannot be changed. In this case, the only way to improve precision is to increase the read time per well. For this reason, a $Z'$ value of around 0.6 is typically sufficient for high throughput primary screening applications; higher values may be desired when results are more critical.

**Note:** The $Z'$ qualification factor is automatically calculated in the **Z'-Factor determination (FP Fluorescein)** template protocol enabled by the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge.

**Performing Measurements**

When the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge is installed in the SpectraMax Paradigm Multi-Mode Detection Platform, detection methods and protocols to perform fluorescence polarization measurements are enabled in Multi-Mode Analysis Software.

When installed in the top read detection cartridge transport, the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge enables the following detection methods:

- Fluorescence Polarization

The SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge enables several default and sample measurement protocols in Multi-Mode Analysis Software for different plate formats and read times which may be used as configured or as examples to create new protocols.

Additional detection methods and protocols using the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge may be created using Multi-Mode Analysis Software.


**Note:** Measurement protocols that use multiple cartridges may also be created and run on the SpectraMax Paradigm Multi-Mode Detection Platform.

**Microplate Recommendations**

The type of microplate and the way it is handled can affect the measurement performance of the instrument. Plate height may not exceed 25 mm. Select a plate type with properties suited for the application and for use with multi-mode detectors. **Table 1-1** lists microplate selection guidelines for each supported measurement type.

Plate handling guidelines include:

- Keep unused plates clean and dry.
- Visually inspect the bottom and rim of the plate before use to make sure it is free of dirt and contaminants.
- Make sure the strips on strip plates are inserted correctly and level with the frame.

**Table 1-1** Microplate selection guidelines

<table>
<thead>
<tr>
<th>Measurement Technique</th>
<th>Microplate Type</th>
<th>Additional Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence Polarization</td>
<td>solid black</td>
<td>When an application specifies a surface treatment, only use plates with the correct treatment.</td>
</tr>
</tbody>
</table>
**Measurement Specifications**

The specifications for measurements using the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge are shown in Table 1-2 on page 21.

**Table 1-2 Measurement specifications**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Cartridge Name</td>
<td>SpectraMax Paradigm Fluorescence Polarization (FP) (Fluorescein) Detection Cartridge</td>
<td>SpectraMax Paradigm Fluorescence Polarization (FP) (Rhodamine) Detection Cartridge</td>
</tr>
<tr>
<td>Short Name</td>
<td>FP-FLUO</td>
<td>FP-RHOD</td>
</tr>
<tr>
<td>Part Number</td>
<td>0200-7009</td>
<td>0200-0710</td>
</tr>
<tr>
<td>Weight</td>
<td>1.5 lbs. (0.7 kg)</td>
<td>1.5 lbs. (0.7 kg)</td>
</tr>
<tr>
<td>Detection Technique</td>
<td>Fluorescence Polarization</td>
<td>Fluorescence Polarization</td>
</tr>
<tr>
<td>Type</td>
<td>Dual emission</td>
<td>Dual emission</td>
</tr>
<tr>
<td>Number of Slots</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Top/Bottom</td>
<td>Top or Bottom</td>
<td>Top or Bottom</td>
</tr>
<tr>
<td>Light Source</td>
<td>LED, ultra high power</td>
<td>LED, ultra high power</td>
</tr>
<tr>
<td>Labels¹</td>
<td>Fluorescein 1 nM</td>
<td>Rhodamine 4 nM</td>
</tr>
</tbody>
</table>
Preventive Maintenance and Troubleshooting

To ensure optimum operation of the instrument, perform the following preventive maintenance procedures as necessary:

- Open the detection cartridge transport only when removing or installing detection cartridges.
- When the detection cartridge is not in use, always keep the red cap in place and store in the detection cartridge box.
- Follow appropriate decontamination procedures as instructed by the laboratory safety officer.
- Contact a Molecular Devices service engineer to inspect the instrument every two years.
- Use a Multi-Mode Validation Plate to regularly validate the performance of the cartridge. Contact Molecular Devices sales or service for more details.
- Respond appropriately to any error messages displayed by Multi-Mode Analysis Software.

**Table 1-2 Measurement specifications**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Limit Top and Plate</td>
<td>384-well plate (75 µL):</td>
<td>384-well plate (75 µL):</td>
</tr>
<tr>
<td>Read Times²</td>
<td>6 mP (1 min read)</td>
<td>8 mP (1 min read)</td>
</tr>
<tr>
<td></td>
<td>3 mP (2 min read)</td>
<td>4 mP (2 min read)</td>
</tr>
<tr>
<td></td>
<td>1536-well plate (8 µL):</td>
<td>1536-well plate (8 µL):</td>
</tr>
<tr>
<td></td>
<td>12 mP (1.5 min read)</td>
<td>12 mP (3 min read)</td>
</tr>
<tr>
<td></td>
<td>6 mP (4 min read)</td>
<td>6 mP (5 min read)</td>
</tr>
<tr>
<td>Validation Plate Tests</td>
<td>test names that end with <strong>-FP-FLUO</strong></td>
<td>test names that end with <strong>-FP-RHOD</strong></td>
</tr>
</tbody>
</table>

1. Additional labels compatible with the excitation and emission wavelengths of the cartridge may also be used.

2. Replicate standard deviation at the label concentration specified under Labels.

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**Preventive Maintenance and Troubleshooting**

To ensure optimum operation of the instrument, perform the following preventive maintenance procedures as necessary:

- Open the detection cartridge transport only when removing or installing detection cartridges.
- When the detection cartridge is not in use, always keep the red cap in place and store in the detection cartridge box.
- Follow appropriate decontamination procedures as instructed by the laboratory safety officer.
- Contact a Molecular Devices service engineer to inspect the instrument every two years.
- Use a Multi-Mode Validation Plate to regularly validate the performance of the cartridge. Contact Molecular Devices sales or service for more details.
- Respond appropriately to any error messages displayed by Multi-Mode Analysis Software.
Validating Performance with the Multi-Mode Validation Plate

The Multi-Mode Validation Plate is used to validate the performance of absorbance, fluorescence, and luminescence measurements performed on the SpectraMax Paradigm Multi-Mode Detection Platform. Validation runs performed with the validation plate validate detection cartridge performance only; factory- and user-defined settings are not changed. The tests which need to be performed to validate performance differ between detection cartridges.

To validate the measurement performance of the SpectraMax Paradigm Fluorescence Polarization (FP) Detection Cartridge, run all validation plate test suites with the detection cartridge short name (see Table 1-2 on page 21) appended to the end of the test name.

**Note:** Refer to the *Multi-Mode Validation Plate User Guide* for information on running tests with the validation plate.
Troubleshooting

**WARNING!** Only officially trained service engineers may perform service procedures on the instrument. Contact a Molecular Devices service engineer when service is required.

Perform the following troubleshooting techniques when necessary. Also refer to “Performing Basic Maintenance and Troubleshooting” in the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*.

**Table 1-3** Troubleshooting the SpectraMax Paradigm Multi-Mode Detection Platform

<table>
<thead>
<tr>
<th>If</th>
<th>Then</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional failure</td>
<td>Install the cartridge in a different detection cartridge slot.</td>
</tr>
<tr>
<td></td>
<td>Contact Molecular Devices Technical Support.</td>
</tr>
<tr>
<td>The Z’ value is too low</td>
<td>• Increase the amount of label in the sample, if acceptable for the assay.</td>
</tr>
<tr>
<td></td>
<td>• Increase the read time per well.</td>
</tr>
<tr>
<td>Detection cartridge fails validation tests with validation plate</td>
<td>Contact Molecular Devices Technical Support.</td>
</tr>
</tbody>
</table>